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RESEARCH ARTICLE

Egg parasitoid complex of the pine processionary moth (*Thaumetopoea pityocampa*) on the Thasos Island, Greece

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Abstract

The egg parasitoid complex of the pine processionary moth (*Thaumetopoea pityocampa*) was surveyed for the first time on the Thasos Island, Greece. A total of 96 egg batches containing 20391 eggs were collected between 06 and 10 of September, 2017 from Aleppo pines (*Pinus halepensis*) at four sites (Skidia, Thimonia, Alyki and Panagia). Four primary parasitoids were identified (*Ooencyrtus pityocampae*, *Baryscapus servadeii*, *Anastatus bifasciatus* and *Trichogramma* sp.), as well as the hyperparasitoid *B. transversalis*. Among the parasitoids groups, *O. pityocampae* was the most common, followed by *B. servadeii*, whilst the number of other species was low. The highest survival rate was reported for three species: *O. pityocampae*, *B. servadeii* and *A. bifasciatus*, while the highest was the mortality in *Trichogramma* sp. All emerged adults of *O. pityocampae* and *B. servadeii* were female specimens and in *A. bifasciatus* – males. The number of females of *B. transversalis* was three times higher than the one of males. *Ooencyrtus pityocampae* and *B. servadeii* were the most important parasitoids of *T. pityocampa*, destroying respectively 27.1% and 9.9% of the host eggs.

Keywords

Thaumetopoea pityocampa, egg parasitoids, survival, impact, Thasos Island

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Introduction

The pine processionary moth, *Thaumetopoea pityocampa* (Denis, Schiffermüller, 1775) (Lepidoptera: Notodontidae) is the most dangerous defoliator in pine forests in the Mediterranean Region. In Greece, the pine stands are mainly composed of natural and exotic species: *Pinus halepensis* Mill., *P. nigra* Arn., *P. radiata* Don, *P. brutia* Ten., *P. pinea* L., *P. pinaster* Aiton and *P. sylvestris* L. *Thaumetopoea pityocampa* attacks both native and exotic pine species, yet a clear preference for *P. halepensis*, *P. nigra* and *P. radiata* was observed (Roques et al., 2015).

The investigations on egg parasitoids of *T. pityocampa* in Greece started more than 50 years ago by Kailidis (1962 a, b), continued over the next decades by Schmidt (1988, 1990), Bellin et al. (1990), Schmidt et al. (1997a), Douma-Petridou et al. (1998), Mirchev et al. (1999a, 2010), Tsankov et al. (1997, 1999), etc. The studies were carried out mainly on biological material from the continental part of the country, collected on the Peloponnese Peninsula (Southern Greece) and in Northern Greece, and as an exception – from the Hydra Island located in the Aegean Sea (Schmidt et al., 1997a). In general, five primary and one secondary egg parasitoids of *T. pityocampa* were identified in Greece: *Ooencyrtus pityocampae* (Mercet, 1921) (Hymenoptera: Encyrtidae), *Baryscapus servadeii* (Domenichini, 1965), *B. transversalis* Graham, 1991 (Hymenoptera: Eulophidae), *Anastatus bifasciatus* (Geoffroy, 1785) (Hymenoptera: Eupelmidae), *Pediobius bruchicida* (Rondani, 1872) (Hymenoptera: Eulophidae) and *Trichogramma* sp. (Hymenoptera: *Trichogramma*tidae) (Mirchev et al., 2010). The hyperparasitoid *B. transversalis* was described from biological material collected in Northern Greece (Graham, 1991). Some main ecological characteristics of the egg stage of *T. pityocampa* on the Thasos Island have been studied (Georgieva et al., 2020), but no data on egg parasitoids of the host have been reported, yet.

The aim of this study was to investigate the main characteristics of the complex of egg parasitoids of *T. pityocampa* from the Thasos Island.

Material and methods

The biological material (96 egg batches of *T. pityocampa* containing 20391 eggs) was collected on 06-10 September, 2017 from Aleppo pines (*P. halepensis*) at four sites on the Thasos Island (Table 1).

The Thasos Island is located in the northern Aegean Sea, at a distance of 6.7 km from the continental part of Greece. With an area of 380.1 km^2, it is the 12th largest island in this country. The terrain is mountainous and is formed by the Ipsario Mt. with the highest peak at 1203 m a.s.l. Three of the studied sites (Skidia, Thimonia and Alyki) are in the south-east part of the island and Panagia – in the north-east one. The climate is characterised by cool summers and mild, wet winters with average annual temperature 17.2 °C and average summer temperature 23.4 °C in July.

Table 1. Main characteristics of the studied areas and collected biological materials

Locality	Geographical coordinates		Altitude, m a.s.l.	Biological material collected		Date of collection
	Latitude	Longitude		Egg batches, n	Eggs, n	
Skidia	40°36'20.32"N	24°43'36.12"E	28	48	10280	06.09.2017
Thimonia	40°36'24.62"N	24°43'14.71"E	11	24	5374	07.09.2017
Alyki	40°36'21.11"N	24°44'26.43"E	18	22	4324	08.09.2017
Panagia	40°43'43.00"N	24°43'50.05"E	351	2	413	10.09.2017

The collected egg batches were transported to the Laboratory of Entomology at the Forest Research Institute in Sofia, Bulgaria. The scales of egg batches were removed and the samples were analysed according to the protocol described in Tsankov et al. (1996). Each egg batch was placed individually in a test tube covered by a cotton stopper and kept under laboratory conditions (20-22 °C). The samples were checked periodically and the emerged parasitoids were removed from the test tubes for identification. In September-October 2018, after the parasitoids completed the emergence, the eggs without openings were dissected and analysed under a stereomicroscope (40×). The parasitoids that had emerged before collection were determined by their meconia and remains according to Schmidt, Kitt (1994), Tanzen, Schmidt (1995), Schmidt et al. (1997a) and Tsankov et al. (1996, 1998). The statistical analyses of the obtained data were carried out with MS Excel 2013 and Statistica for Windows v. 12.

Results

Four primary parasitoids (*Ooencyrtus pityocampae*, *Baryscapus servadeii*, *Anastatus bifasciatus*, and *Trichogramma* sp.) and one hyperparasitoid (*B. transversalis*) were reared from the eggs of *T. pityocampa* collected on the Thasos Island (Fig. 1).

At three of the studied sites (Skidia, Thimonia and Alyki), the most numerous was the polyphagous *O. pityocampae* with 63.8-75.5% of all emerged specimens (Fig. 1). The single exception was Panagia (with 6.8%), in which only two egg batches were studied. In the other three samples, the ratio between the two most abundant parasitoids, *O. pityocampae* and *B. servadeii*, varied between 2:1 and 3:1. The number of the polyphagous *A. bifasciatus* was more significant only in Thimonia (1.1%), while at the other sites, it was absent or a single specimen was established. *Trichogramma* sp. occupied 3.4-9.3% of the parasitoid complex of *T. pityocampa* with an average of 6.0% for all studied sites. The two most numerous parasitoids of *T. pityocampa*, *O. pityocampae* and *B. servadeii*, were found respectively in 96.9% and 81.3% of the egg batches of the host (Table 2). *Trichogramma* sp. was recorded from 58.3% of the egg batches of the pine processionary moth.

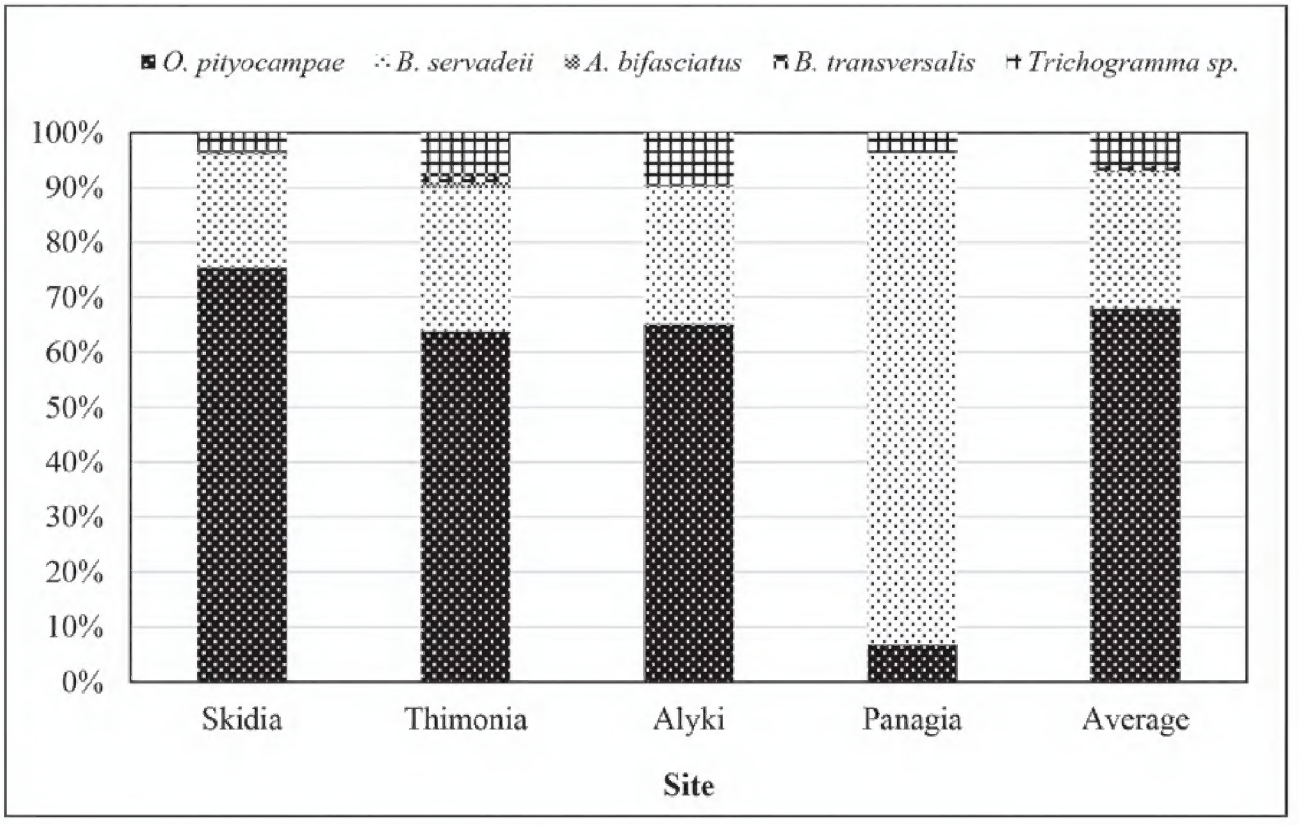

Figure 1. Percentage of egg parasitoids of *T. pityocampa* at the studied sites on the Thasos Island

High survival was established in three primary parasitoids. The mortality of *O. pityocampae* and *B. servadeii* at different stages of their development in the host eggs was below 5.0%, and no dead individuals of *A. bifasciatus* were recorded. The mortality of the polyembryonic *Trichogramma* sp. was significantly higher (76.8%), and of the hyperparasitoid *B. transversalis* was 11.1%. It should be noted that 792 specimens (8.9%) of the parasitoids had died at an early stage in their development and their identification was impossible.

All emerged individuals of *O. pityocampae* and *B. servadeii* were female, while only male specimens were recorded for *A. bifasciatus*. In the hyperparasitoid *B. transversalis*, the ratio of females to males was 3:1. A significant part of the adults of *O. pityocampae* (49.3%) and *B. servadeii* (29.2%) had emerged before the date of sample collection. On the other hand, all adults of *A. bifasciatus* and *B. transversalis* emerged after the collection.

The mortality of *T. pityocampa* caused by egg parasitoids in the studied areas ranged between 37.3% (Skidia) and 53.5% (Thimonia), with an average of 43.4% for all studied sites (Fig. 2).

Ooencyrtus pityocampae and *B. servadeii* were the most important parasitoids of *T. pityocampa*, destroying an average of 27.1% and 9.9% of the eggs, respectively. Unidentified dead parasitoid larvae were found in 3.9% of the host eggs. The rest of the parasitoids occurred in relatively low numbers as follows: *A. bifasciatus* (0.2%) and *Trichogramma* sp. (2.4%). They revealed low impact as regulating agents of the pine processionary moth at the egg stage.

The hyperparasitoid *B. transversalis* parasitized 0.9% of *B. servadeii* and *O. pityocampae* on the Thasos Island.

Table 2. Characteristics of egg parasitoids of *T. pityocampa* at the different localities

Parameters	Sites									
	Skidia		Thimonia		Alyki		Panagia		Total	
	N	%	n	%	n	%	n	%	n	%
Ooencyrtus pityocampae	2643	100.0	1674	100.0	1201	100.0	11	100.0	5529	100.0
Emerged before collection of egg batches	1228	46.5	843	50.3	649	54.1	7	63.6	2727	49.3
Emerged after collection of egg batches	1263	47.8	746	44.6	499	41.5	-	0.0	2508	45.4
Adults died in eggs	152	5.7	85	5.1	53	4.4	4	36.4	294	5.3
Egg batches with *O. pityocampae*	46	95.8	24	100.0	22	100.0	1	50.0	93	96.9
Baryscapus servadeii	716	100.0	686	100.0	463	100.0	144	100.0	2009	100.0
Emerged before collection of egg batches	141	19.7	248	36.1	125	27.0	72	50.0	586	29.2
Emerged after collection of egg batches	562	78.5	412	60.1	323	69.8	72	50.0	1369	68.1
Adults died in eggs	13	1.8	26	3.8	15	3.2	-	0.0	54	2.7
Egg batches with *B. servadeii*	33	68.8	22	91.7	21	95.5	2	100.0	78	81.3
Anastatus bifasciatus	1	100.0	30	100.0	-	0.0	-	0.0	31	100.0
Emerged after collection of egg batches	1	100.0	30	100.0	-	0.0	-	0.0	31	100.0
Egg batches with *A. bifasciatus*	1	2.1	2	8.3	-	0.0	-	0.0	3	3.1
Baryscapus transversalis	23	100.0	42	100.0	7	100.0	-	0.0	72	100.0
Emerged after collection of egg batches, ♀	14	60.9	33	78.6	1	14.3	-	0.0	48	66.7
Emerged after collection of egg batches, ♂	5	21.7	7	16.7	4	57.1	-	0.0	16	22.2
Adults died in eggs	4	17.4	2	4.7	2	28.6	-	0.0	8	11.1
Egg batches with *B. transversalis*	8	16.7	10	41.7	6	27.3	-	0.0	24	25.0
Trichogramma sp.	119	100.0	194	100.0	172	100.0	6	100.0	491	100.0
Emerged before collection of egg batches	27	22.7	50	25.8	29	16.9	6	100.0	112	22.8
Emerged after collection of egg batches	-	0.0	-	0.0	2	1.1	-	0.0	2	0.4
Eggs with dead adults	92	77.3	144	74.2	141	82.0	-	0.0	377	76.8
Egg batches with *Trichogramma* sp.	26	54.2	16	66.7	13	59.1	1	50.0	56	58.3
Undetermined larvae of parasitoids	358	100.0	292	100.0	131	100.0	11	100.0	792	100.0

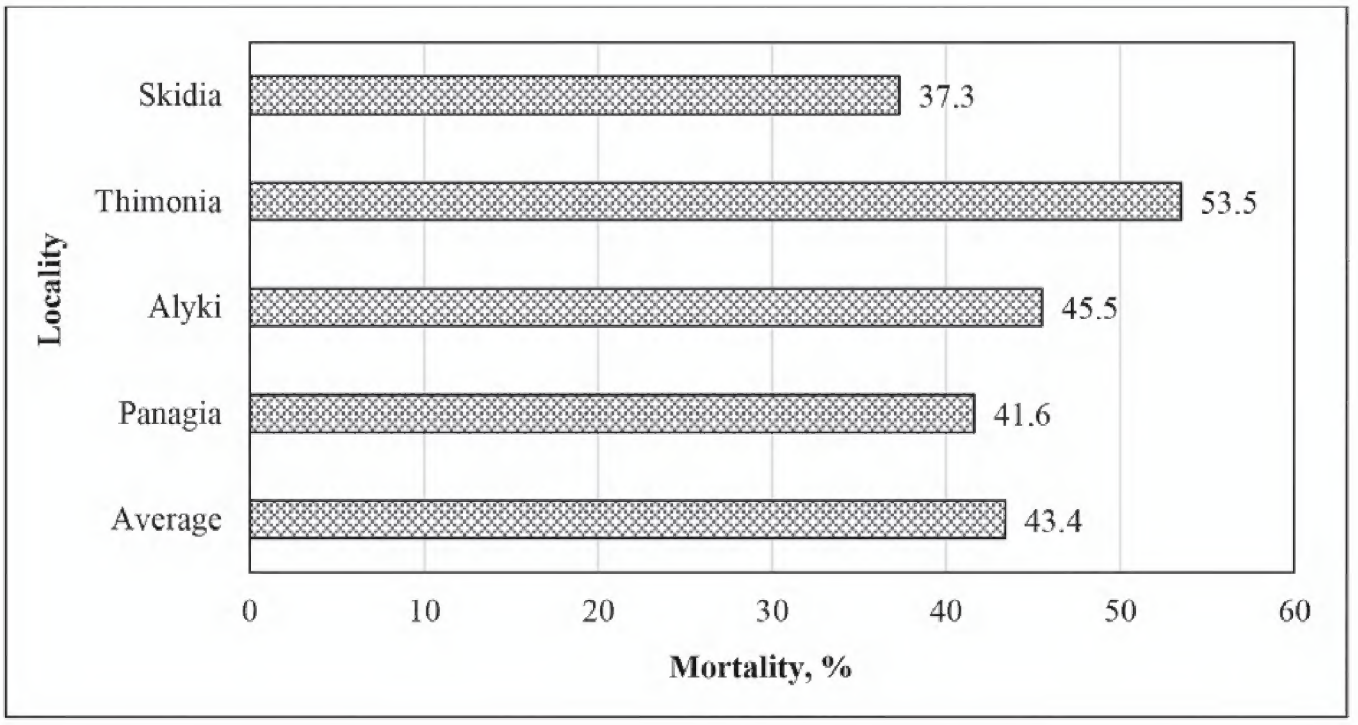

Figure 2. Mortality of *T. pityocampa* caused by egg parasitoids in different localities of the Thasos Island

Discussion

In one of the first investigations on egg parasitoids of *T. pityocampa* in Greece, Kailidis (1962b) reported two species: *O. pityocampae* and *Tetrastichus* sp. from the Athens, Thessaloniki and the Attica Regions. In subsequent studies on the Peloponnese Peninsula and in Northern Greece, *B. servadeii*, *A. bifasciatus* and *Trichogramma* sp. were added to the species composition of the primary egg parasitoids of *T. pityocampa* in the country (Schmidt, 1988, 1990; Bellin et al., 1990). In addition, Bellin et al. (1990) reported *Baryscapus* sp. near *servadeii*, which has been described later as *B. transversalis* (Graham, 1991). Tsankov et al. (1999) found *Pediobius* sp. as an egg parasitoid of *T. pityocampa* in the Patra Region (Peloponnese Peninsula). Recently, Mirchev et al. (2010) reported *Pediobius bruchicida* as a parasitoid of the host in the Drama and the Kastania Regions in Northern Greece.

Outside the continental part of Greece, three egg parasitoids of *T. pityocampa* were established on the Hydra Island, with a great superiority in the number of *O. pityocampae* (78.3%), followed by *B. servadeii* (18.7%) and by *B. transversalis* (3.0%) (Schmidt et al., 1997a). Bellin et al. (1990) found a significant difference in the ratio between the two most abundant primary parasitoids of the host in Greece: in the Kalogria Region (North-western Peloponnese Peninsula). According the authors, the share of *O. pityocampae* and *B. servadeii* was almost equal – 52.7% and 47.3%, respectively, while in the Kasandra Region (Northern Greece), the number of *O. pityocampae* was 3.8-6.2 times lower. In 1997 in Kalogria, the number of *B. servadeii* was nearly three times higher than the number of *O. pityocampae*, but in the Athens Region (Southern Greece), *O. pityocampae* predominated (Mirchev et al., 2010). In this study, *O. pityocampae* predominated in three of the four studied

localities on the Thasos Island. These results differ significantly from data obtained for other regions in Northern Greece: *O. pityocampae* was more numerous only in the Thessaloniki Region, while at other sites (Drama, Kastania and Asprovalta), a higher share of *B. servadeii* in the parasitoid complex of the pine processionary moth was reported (Mirchev et al., 2010).

There are several hypotheses that seek to explain the diversity of egg parasitoids of *T. pityocampa* in different areas. Masutti (1964) has pointed that the representatives of family Eulophidae are more plastic and develop successfully in areas with temperatures above 30 °C, which are not favourable for the development of *O. pityocampae*. This hypothesis has been supported by the fact that in Italy, the oligophagous *B. servadeii* is predominant in the warmer regions of the central and southern parts of the country, but it is unexpected that the species has not been found on the Sicily Island and in the pine forests of the Abruzzo National Park at low altitudes (Tiberi, 1990). As concerns polyphagous parasitoids, such as *O. pityocampae*, it has been suggested that the floristic richness of the habitats is a prerequisite for the high parasitoid numbers, due to the favourable conditions for development of alternative hosts (Mirchev, 2005).

On the Thasos Island, all emerged adults of *O. pityocampae* and *B. servadeii* were female specimens. A similar pattern (only emerged females of *O. pityocampae* and up to 2.2% of emerged males of *B. servadeii*) has been previously found in other areas of the range of *T. pityocampa*: Algeria (Tsankov et al., 1995); Morocco (Schmidt et al., 1997b); Albania (Mirchev et al., 1999b); Portugal (Mirchev, Tsankov, 2000); Macedonia (Tsankov et al., 2006); Turkey (Mirchev et al., 2004), Greece (Mirchev et al., 2010). In this study, all emerged adults of *A. bifasciatus* were male specimens. The development of females of *A. bifasciatus* in egg batches of the pine processionary moth usually is very rare, but in the Eastern Rhodopes in Bulgaria, 13.8% female specimens of the parasitoid were reared from *T. pityocampa* (Mirchev et al., 1998).

The ratio of females to males of the hyperparasitoid *B. transversalis* on the Thasos Island was 3:1. Similar ratio (approximately 2:1) has been reported for the Hydra Island (Schmidt et al., 1997a) and for the Peloponnese Peninsula (Tsankov et al., 1999). In Northern Greece, the share of *B. transversalis* in the parasitoid complex of *T. pityocampa* is below 3.0-6.3% (Bellin et al., 1990; Mirchev et al., 2010). In this study, the relative share of *B. transversalis* was very low: between 0 and 1.6%, with an average of 0.9%. The high values of egg parasitism of *T. pityocampa* at the studied sites on the Thasos Island (37.7-53.5%) is probably due to the low suppressive impact of the hyperparasitoid on *O. pityocampae* and *B. servadeii*.

In conclusion, it should be noted that this is the first study of egg parasitoids of *T. pityocampa* on the Thassos Island. It extends our knowledge about the biology of the species and their effects on the host.

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